We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 21

Human Embryonic Stem Cells and Induced Pluripotent Stem Cells: The Promising Tools for Insulin-Producing Cell Generation

Wilairat Leeanansaksiri,
Piyaporn Rattananinsruang and
Chavaboon Dechsukhum

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62850

Abstract

Diabetes mellitus, a disease with abnormally high level of blood glucose, can cause a wide range of chronic complications that affect almost every parts of the body. The major goal of diabetes treatments is to control elevated blood glucose without causing abnormally low levels of blood glucose. Despite islet transplantation provided endogenous insulin secretion in individuals with diabetes, the scarcity of cadaveric donors for pancreatic β-cell still remains a major obstacle. In this regard, the needs for an unlimited supply for cell replacement strategy have led to explore the way of generating insulin-producing cells to use in the disease treatment. Human embryonic stem cells (hESCs) offer a source to produce the desired kind of cell. Currently, several researchers achieved insulin-producing cells from hESCs using a multistep differentiation protocols, growth factors, and/or chemical compounds. In this review, we summarized the hESCs derivation, culture methods, and characteristics of hESCs. We also emphasized on the current methods for direct differentiation of hESCs into embryoid bodies (EBs) and toward insulin-producing cells, characterization of these insulin-producing cells, and the limitation of hESCs. Since the discovery of induced pluripotent stem cells (iPSCs), which have similar properties to hESCs but less ethical issues than hESCs, can be created directly from somatic cells that hold great promise as the therapeutic source for developing cell-based therapy. Herein, the methods to produce iPSC-derived insulin-producing cells are also discussed. Moreover, the encapsulation technology which is a powerful tool for accelerate hESCs and iPSCs applications in medicine which provide a new avenue for diabetes treatment in the future is also included in this review. Understanding the basic knowledge of hESCs and iPSCs, their differentiation capability toward insulin-producing cells will stimulate

more therapeutic value of hESCs and iPSCs for diabetic treatments, drug screening, and regenerative medicine.

Keywords: diabetes mellitus, human embryonic stem cells, insulin-producing cells, insulin, encapsulation, induced pluripotent stem cells

1. Introduction

Diabetes mellitus is one of the most common chronic diseases that threaten the health and health economics worldwide [1–3]. The disease and cases management interventions improve short- and long-term health, and/or economic outcomes of patients are improving health and quality of their lives. General treatments include weight reduction, a diabetes diet, and exercise which are used to control diabetes [4, 5]. When these treatments fail to control the elevated blood glucose, oral medications and insulin injection therapy will be applied in the treatment strategy, respectively. Insulin therapy has the potential to improve symptoms, enhancing quality of life and provide a sense of well-being [6–8]. However, the intensive insulin treatment can cause hypoglycemia [9–11]. Therefore, transplantation of high effective insulin-producing cells containing physiological regulation of blood glucose level is critical choice of treatment. At present, there are three major cell-based therapy approaches to restore insulin-producing cells in diabetes patients: 1) direct transplantation of donor islet and pancreatic cells to patient 2) activation pancreatic progenitors residing in islet mass into insulin-producing cells (neogenesis) and transplantation, and 3) stem cell approach by differentiation pluripotent stem cells into insulin-secreted cells and then transplantation. The direct transplantation of islet cells and neogenesis methods have demonstrated normoglycemia in the absence of exogenous insulin therapy [12–14]. Nevertheless, the limitations of islet cell replacement are the following factors: non-functioning of isolated islets, the small number of transplanted islets, the immunogenicity of isolated islets lead to immune rejection, transplantation to inappropriate sites, recurrence of auto-immunity in the transplanted islets, and immunosuppression [15–18]. The third method, stem cell approach: Pluripotent stem cells including hESCs and iPSCs are the main targets for insulin-producing cell induction. These two sources of stem cells could provide limitless sources of cells for pancreatic β-cell replacement strategy. Therefore, these research areas have led researchers to explore the way of generating effective insulin-producing cells for diabetes treatment. In addition, the hESCs and iPSCs contain the higher differentiation capacity than adult stem cells [19–21].

Herein, we review the current knowledge of hESCs and iPSCs, followed by the directed differentiation of these cells toward the functional insulin-producing cells. In addition, the encapsulation technology, a powerful tool for accelerate hESCs and iPSCs applications in medicine, is also included in this review. We also summarize and discuss evidence that both hESCs and iPSCs are promising cell sources for future diabetes treatment.

http://dx.doi.org/10.5772/62850

2. Human embryonic stem cells

2.1. Derivation, culture, and characterization of hESCs

In the early era of hESCs research, the hESCs are isolated from the inner cell mass (ICM) of blastocyst-stage embryos by immunosurgery or mechanically methods. However, this process involves with animal-derived substances such as mouse antibodies and guinea pig complement [22, 23]. Alternatively, the use of Tyrode's acid for the removal of zona pellucida and mechanical isolation of ICM can serve as a potentially useful method for the establishment of hESCs line in the present time. Indeed, this technique also implies that the blastocyst could not contact with animal-derived pronase, antibodies, and complement factors [22]. The hESCs lines can be maintained in an undifferentiated or pluripotency state *in vitro* for prolonged periods of time. The potential of hESCs to differentiate into representing ectoderm, mesoderm, and endoderm derivatives has generated the possible use of hESCs in therapeutic applications [24]. The derivation process involves culturing of the ICM of blastocyst stage, induce to proliferate and differentiate into desired cell types [25]. The first successful derivation of hESCs was isolated from the ICM of human blastocyst and placed on mitotically inactivated murine feeder cells [26].

There are several methods have been reported for culture of undifferentiated hESCs *in vitro* such as culture the undifferentiated hESCs on feeder layers, for example, mouse embryonic fibroblast (MEF) or laminin- or Matrigel-coated-plastic surface with MEF conditioned medium. However, these methods possibly transfer harmful animal pathogens to human transplant recipients in clinical application [27]. Therefore, human feeder layers are used for hESCs culture instead, for example, human adult marrow cells, human fetal muscle (FM), human adult skin (AS), commercial human fetal skin (FS; D551/CCL-10, American Type Culture Collection [ATCC]), human adult uterine endometrial cells (hUECs), human adult breast parenchymal cells (hBPCs), and embryonic fibroblasts (hEFs). They are capable to support undifferentiated stage and proliferation state of hESCs [28–31]. Conditioned medium from hESCs-derived fibroblasts (hESC-dFs) also efficiently supports growth of hESCs in feeder-free culture systems [32]. Moreover, a three-dimensional (3D) porous natural polymer scaffolds (chitosan and alginate) effectively support self-renewal of hESCs without the need of feeder cells or conditioned medium [33]. Recent study has demonstrated that a defined engineered 3D microfiber system allows adequate propagation and cryopreservation of hESCs under feeder-free chemically defined conditions [34]. However, these culture conditions still have the ingredients from animal such as fetal bovine serum (FBS) and bovine albumin that contain in culture medium. For the clinical potential in cell replacement therapy, differentiated cells from hESCs should be cultured in xeno-free systems [27, 31]. Interestingly, Chen et al. reported that the suspension culture system under defined and serum-free conditions provides a powerful approach for scale-up expansion of hESCs. It was demonstrated that cell banks of several hESCs lines are generated from this system under current good manufacturing procedures (cGMP) or cGMP-equivalent conditions [35].

Characterization of hESCs lines can be achieved by both cellular and molecular analysis. Cellular characterizations can be determined by (1) morphologies of hESCs colonies: form flat

and compact colonies with distinct cell borders, (2) morphologies of hESCs have a high ratio of nucleus to cytoplasm and have prominent nucleoli, (3) the hESCs exhibit high levels of telomerase activity and show normal karyotype, (4) most of the cells can be subcultured after freezing, thawing, and replating, (5) the cells can be differentiated into a variety of cell types both *in vitro* and *in vivo* conditions, and (6) the cells can generate teratoma in animal model or *in vivo*. The general molecular properties of hESCs lines can be examined by the expression of several transcripts, for example, the stage-specific antigens (SSEA-3 and SSEA-4), the glycoproteins tumor recognition antigen (TRA-1-60, TRA-1-81 and TRA-2-54), germ cell tumor marker (GCTM-2), trophoblast giant (TG343 and 30), cluster of differentiation (CD9 and 133), Octamer-4 (Oct4), Nanog, SRY-box-containing gene 2 (*Sox2*), teratocarcinoma-derived growth factor 1 (Tdgf1), left–right determination factor 2 (LeftyA), RNA exonuclease 1 (Rex-1), Stellar, Dazl, Nanos 1, pumilio gene (*Pum 1* and *2*), growth differentiation factor-3 (Gdf3), thymus cell antigen 1 (Thy-1), and alkaline phosphatase [25, 36]. Other markers that are common to characterize hESCs are following: SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, Oct4, and alkaline phosphatase. Nevertheless, there are differences between hESCs in their pluripotency or the genetic profile under the same conditions, their potential for large-scale culture and growth under feeder-free protocols, or their ability to form teratoma after injection into severe combined immunodeficiency (SCID) mice. Moreover, their capacity to differentiate into different cell types under *in vitro* conditions is variable [25, 36]. It is important to note that the difference in various hESCs lines is useful for the scientists to choose the appropriate hESCs line for their research.

2.2. Differentiation of hESCs into embryoid bodies (EBs) and definitive endoderm (DE)

To differentiate hESCs into particular cell lineages *in vitro*, the formation of embryoid bodies (EBs) represents an important step to generate three germ layers; ectoderm, mesoderm, and endoderm. The EBs will be then derive into tissue-specific progenitor cells and toward the desire final differentiated lineages. It should be noted that hESCs are able to differentiate through EBs parallels embryonic development due to the EBs recapitulates events during embryogenesis [37]. The use of EBs to produce a variety of desired cell types represents an exciting approach for therapeutic applications.

For the production of EBs, several methods have been designed. The first method: Methylcellulose (MC) method developed to form EB from single embryonic stem cell but has limitation to use EBs for medical application due to the contamination of methylcellulose. The second method, hanging drop (HD) method has been widely used to generate EBs, and further differentiate into a variety of cell types can be applied into other xenofree or chemically defined medium culture protocols that suitable for human therapeutic applications. However, it is a troublesome multiple steps methods. Moreover, it is hardly exchange that the medium for a drop and the observations of forming EBs in drops by direct microscopic is difficult [38]. Therefore, the third method has been developed called suspension culture method—the obtained EBs tends to be more heterogeneous in size and shape because their self-organized aggregation in culture. The heterogeneity of EBs structures may influence cell fate differentiation [38, 39].

http://dx.doi.org/10.5772/62850

In fact, improvement of the definitive endoderm (DE) layer development will increase the successful rate of insulin-producing cell generation from pluripotent stem cells. Therefore, there are several factors have been used to activation of DE formation, for example, activin A, GDF8, Wnt3a, bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), inhibition of PI3K/Akt, and chemical inhibition of GSK3β [40]. In addition, collagen type 1 could improve the differentiation of hESCs into definitive endoderm [41].

2.3. Differentiation of hESCs into insulin-producing cells

At present time, cell-based therapy for diabetes is mostly target to type I diabetes. It has been described that islet-like clusters derive hESCs differentiation can be achieved in multistep procedures (nestin-positive protocol): (i) formation of EBs (stage 1), (ii) selective differentiation of cell populations expressing nestin using fetal calf serum depletion and culture with ITSF (stage 2), (iii) proliferation and maintenance of precursor cells (stage 3), and (iv) the differentiation induction and maintenance of insulin-positive cells (stage 4). The resultant cells are positive for dithizone (DTZ), a zinc-chelating agent known to selectively stain pancreatic β-cells, and are immunoreactive for antibodies against insulin, glucagons, and C-peptide. Insulin and other pancreatic β-cell-related genes such as glucagon, somatostatin, KIR6.2 and SUR1, IAPP, Isl1, PC1/3, PC2, GK, Nkx6.1, GLUT2, and Pax4 are expressed in the differentiating cells. The results indicated that differentiated cells can express genes involved in the β-cell differentiation pathway [42].

Moreover, insulin-producing islet-like clusters (ILCs) are generated from hESCs according to the method (definitive endoderm-based protocols) developed by Jiang and colleagues [43] who culture hESC lines under feeder-free conditions and direct differentiation toward ILCs by using a multistep, serum-free protocol. The 36-day differentiation protocol consists of four stages which included definitive endoderm induction (stage 1), pancreatic endoderm formation (stage 2), endocrine induction (stage 3), and islet-like clusters maturation (stage 4). The hESCs generate definitive endoderm coexpressing CXCR4 and Sox17, and CXCR4 and Foxa2 when treated with sodium butyrate and activin A. The Pdx1-expressing pancreatic endoderm is then induced by the addition of bFGF, EGF, noggin, and B27 supplement. Following withdrawal of bFGF, these cells are allowed to develop pancreatic endocrine cells. Gene expression analysis shows that pancreatic endoderm cells also start to express other pancreas-related genes such as HlxB9, Ptf1a, Ngn3, and Nkx6.1. Upon further differentiation of Pdx1-positive cell clusters to day 36, immunocytochemical-staining data demonstrated that the C-peptide-, glucagon-, and somatostatin-positive cells were predominantly localized in the small bud-like clusters as well as in some of the smaller ILCs. In addition, the ILCs generated by this protocol are able to secrete C-peptide in response to 20 mM glucose [43].

Based on the differentiation protocol, the nestin-positive progenitor-based and definitive endoderm-based protocols are successful in generating insulin-producing cells from hESCs. However, there is still some debate on the therapeutic potential between the cells obtained from these two protocols. Therefore, other approaches are developed. Bruin and colleagues implemented an approach in which hESCs were differentiated into fetal-like pancreatic cells *in vitro*. Taking an approach, this group set out to replace activinA/wnt3A with GDF8/GSK3β

inhibitor to enhance efficiency of definitive endoderm production. This study achieved to generate insulin-producing cells and also revealed several key features of polyhormonal insulin-positive cells that differ from mature pancreatic β-cells, including defects in glucose transporter expression, KATP channel function, and prohormone-processing enzymes [44].

The strategies for differentiation of hESCs into insulin-producing cells also have been demonstrated by many research groups. They demonstrated that the insulin-producing cells are expressed the markers associated with pancreatic β-cell differentiation pathway, able to produce and secrete insulin in response to glucose concentration [42, 45–49]. The spontaneous differentiation of insulin-producing cells can also be observed with undifferentiated hESCs colonies when hESCs are propagated on a feeder layer of MEFs [45]. It has been demonstrated that *in vitro* differentiation in suspension culture results in the formation of discrete embryoid bodies (EBs) and a more consistent pattern of nestin-positive progenitors which possibly are insulin-producing progenitors [49] (**Figure 1**).

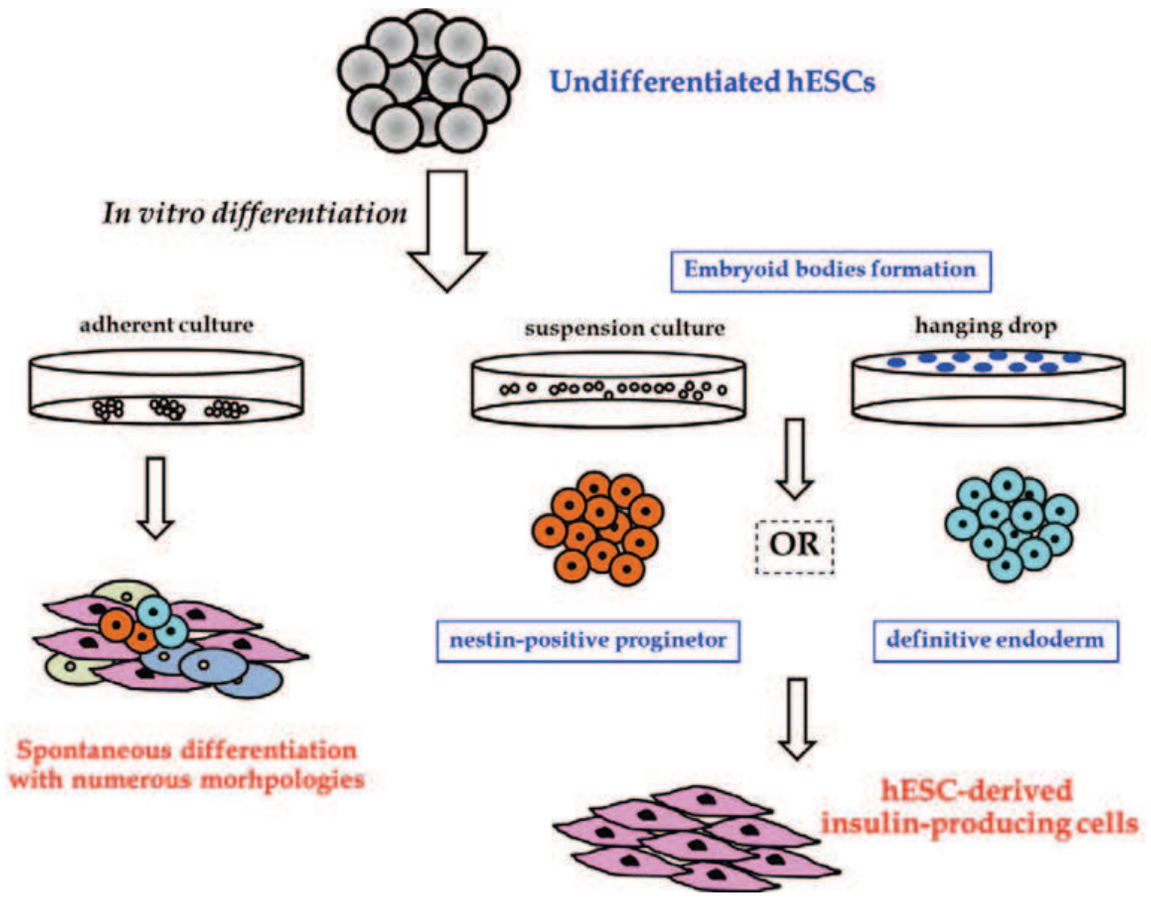

Figure 1. Summary of *in vitro* differentiation protocol for derivation of insulin-producing cells from hESCs. The directions of hESCs into insulin-producing cells are based on the variety of experimental approaches. During *in vitro* differentiation hESCs, the procedures were performed through a multistep including adherent culture, suspension culture, and hanging drop for generating the precursor cells. Cells grown under adherent conditions displayed a pleiotropic pattern with numerous morphologies. In addition, the differentiation of hESCs into insulin-producing cells was successfully induced by the nestin-positive progenitor-based and definitive endoderm-based protocols [43–46, 49].

http://dx.doi.org/10.5772/62850

Recently, it has been shown that maturation of stem cell into beta-cells can be driven by the expression of peptide hormone called urocortin 3 which also served as islet cell maturation marker [50]. In addition, the scalable differentiation protocol also able to generate several millions of glucose-responsive β-cells from hESCs [51]. Interestingly, it has been revealed that acceleration of Cdk4-cyclin D complex in the cell cycle of hESCs also play a potential key role in cell fate decision of hESCs differentiation into insulin-producing cell [52].

Although several authors achieved to demonstrate the rapid progress for possible treatment of diabetes using insulin-producing cells generated from hESCs, there are some crucial aspects need to be concerned. Based on this regard, the culture should be performed in xeno-free system in order to further develop potential medical applications. Moreover, the contamination of undifferentiated hESCs in the culture of insulin-producing cell induction needs to be removed before it can be applied for therapeutic purposes to eliminate the teratoma formation. The immune rejection aspect is also need to be assessed. For examples, during differentiation stage, the major histocompatibility complex (MHC) is up-regulated, leading to the non-self-proteins expression on the graft cells which may result in immune rejection of the graft in the absence of immunosuppressive therapy [25]. In addition, the expression of high level of telomerase activity can lead to teratoma formation after injection which should be noted [53]. Furthermore, prolonged growth of hESCs and differentiation of these cells into any stages of beta-cells development *in vitro* may also cause chromosomal aberrations. Therefore, complete characterization of hESC lines, insulin-producing cells, or other pancreatic cell stages that will be applied for therapeutic purposes need to be well analysis, for example, their molecular status, a continuous genetic, and chromosomal features.

2.4. Transplantation of hESC-derived insulin-producing cells

In order to gain insights into the human condition, the function of hESC-derived insulin-producing cells should be achieved *in vivo*. Also, many research groups have tried to transplant insulin-producing cells derived from hESCs into animal models of diabetes mellitus. For instance, the transplantation of hESC-derived ILC under the kidney capsule of streptozotocin (STZ)-induced diabetic immuno-incompetent mice exerted functional benefits. The grafted cells continued to contain cells that were shown the ability to secrete human C-peptide in response to an oral bolus of glucose. In addition, the transplanted ILCs could promote the mean survival of recipients as compared to controls, which were transplanted with human fibroblast cells [54]. Hua and colleagues reported the protocol for differentiating hESCs into pancreatic insulin-producing cells and transplanted the cells into severe combined immunodeficient (SCID)/non-obese diabetic (NOD) mice to assess graft survival and function. The terminally differentiated cells were glucose-responsive and expressed C-peptide, similar to pancreatic islets. When transplanted into the epididymal fat pad of SCID/NOD mice, these cells were capable of correcting hyperglycemia for ≥ 8 weeks. Notably, none of the treated animals developed tumors [55]. Alternatively, the function of pancreatic endoderm or pancreatic progenitors derived from hESCs has been observed following transplantation into diabetic models. Recent study has demonstrated that the engrafted hESC-derived pancreatic endoderm generated functional endocrine cells when implanted in the epididymal fat pad of

SCID/Beige mice. The grafted cells exhibited appropriate expression of pancreatic transcription factors, expressed prohormone-processing enzymes, and contained mature, homogeneous endocrine secretory granules. Moreover, the engrafted hESC-derived endocrine cells regulated glucose homeostasis in the host by synthesized and released insulin in response to glucose levels [56]. Rezania et al. provided evidence that hESC-derived pancreatic progenitor cells successfully matured into functional islets *in vivo* and controlled glycemia of STZ-treated immunodeficient mice. The study demonstrated that the differentiation protocol has generated a highly enriched Pdx1+ pancreatic progenitor cell population *in vitro* without cell sorting. These progenitor cells were remarkably developed similar to human fetal pancreas development and resulted in the formation of insulin-producing cells that closely resembled matured human β-cells. The implanted cells contributed to protect mice against STZ-induced diabetes with robust glucose-stimulated human C-peptide secretion *in vivo* [57]. Currently, cellular encapsulation has been provided immunoprotection in host with the potential to reduce or eliminate the need for chronic immunosuppression. In a recent study, Kirk and colleagues used a bilaminar device (Theracyte) to investigate the kinetics of cellular engraftment and the maturation dynamics of hESC-derived pancreatic epithelium (PE). They found that *in vitro* derived hESC-PE cells generated through glucose-responsive insulin-producing cells inside an encapsulation device with no increase in cell mass and without cell escape. The encapsulated hESC-derived PE exhibited full physiological function *in vivo* and resulted in amelioration of alloxan-induced diabetes following implantation [58]. Despite promising findings in a test of therapeutic potential, both safety and efficacy of the hESC-based therapy for insulin-dependent diabetes must be further investigated.

3. Induced pluripotent stem cells

3.1. The generation of iPSCs

The induced pluripotent stem cells (iPSCs) also provided an alternative approach to produce autologous cell-based therapy (**Figure 2**). The iPSCs have been shown the properties similar to hESCs including morphology, self-renewal capacity, gene expression profiles, and retained a normal karyotype. The differentiation potential of these cells would allow researchers to study disease mechanisms, drug screening and provide another autologous cell sources for transplantation [59–61].

Based on the knowledge of transcriptional regulators that maintain the stem cell state, researchers have been developed a technique that can be reprogrammed adult cells into pluripotent stage. In 2006, Takahashi and Yamanaka discovered that the introduction of four transcription factors (Oct4, Sox2, c-Myc, and Klf4) could induce pluripotency in mouse embryonic or adult fibroblasts. These iPSCs exhibited the morphology and growth properties of ESCs and expressed ESC marker genes. The resultant cells resulted in tumors containing a variety of tissues from all three germ layers when transplantation into nude mice. Consequently, Yu and colleagues designed a reprogramming strategy in which a set of four factors (Oct4, Sox2, Nanog, and Lin28) were sufficient to induce pluripotency in human

http://dx.doi.org/10.5772/62850

somatic cells. These cells displayed normal karyotypes, expressed telomerase activity, expressed the hESC surface markers/genes, and maintained the developmental potential to differentiate into advanced derivatives of all three primary germ layers [62]. Additionally, it has been found that the miR-302 family is specifically expressed in undifferentiated ESCs. The miR-302 has a role in regulating ESC pluripotency and differentiation [63, 64]. Also, several studies have demonstrated that miR-302 can directly reprogram somatic cells. Lin et al. reported that miR-302 inhibits stem cell tumorigenicity by enhancing G1 phase arrests pathway [65]. This evidence suggests that the miR-302-reprogrammed iPSCs may provide the potential applications of iPSC technology.

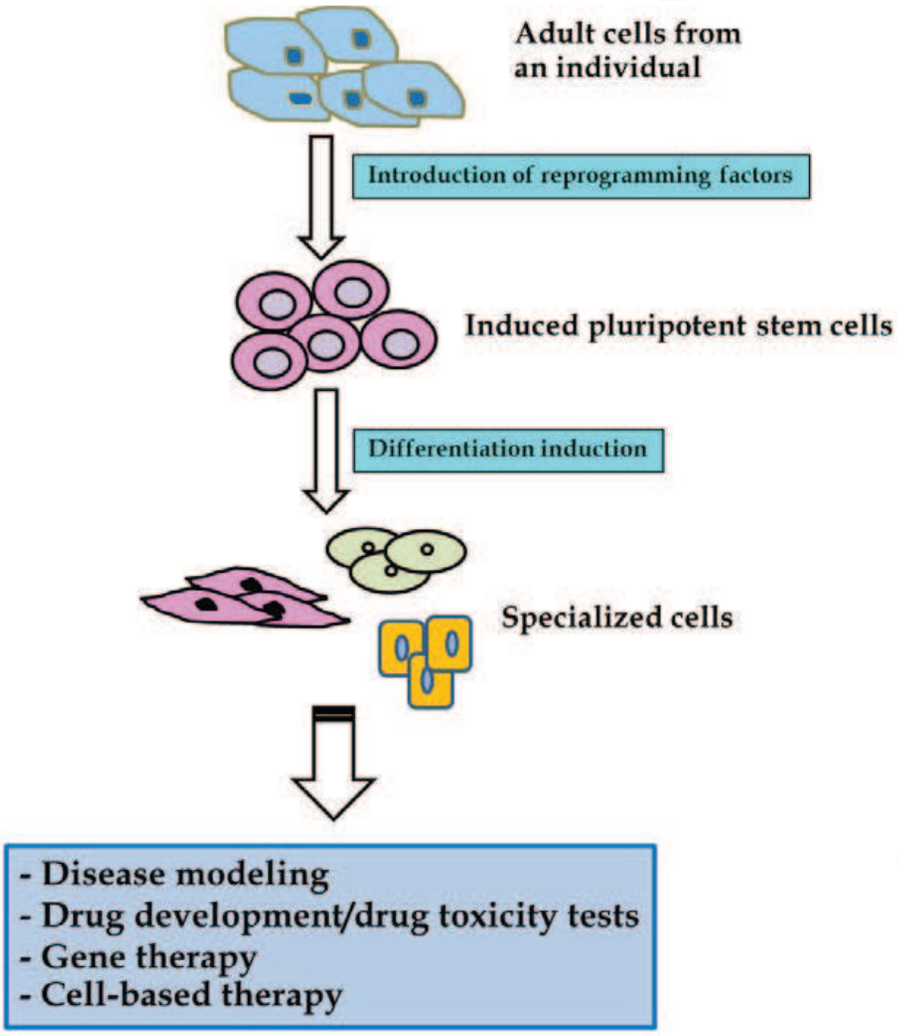

Figure 2. Induced pluripotent stem cells (iPSCs) and the potential use in regenerative medicine. Adult cells or somatic cells can be reprogrammed into pluripotent stem cells by introducing a specific set of reprogramming factors. These iPSCs can then be differentiated to specialized cell types, which can be used as a clinical tool for disease modeling, drug development/drug toxicity tests, gene therapy, and cell-based therapy.

The approach to reprogram somatic cells involved the use of genome-integrating retroviruses to transfer transcription factors. However, the usage of integrating retroviral vector has

major limitation due to the potential risk relating to tumor formation. Thus, researchers have also used the different strategies with non-integrating system that provided the solution to make it clinically applicable [66]. Although the reprogramming efficiencies with non-integrating method are lower (~0.001%) than those achieved with integrating method (0.1–1%), this method increases the safety of generating and using iPSCs [67]. Alternative methods have been developed to avoid genetic modification as the reprogramming proteins, or mRNA was delivered directly into the cells. This has been successfully demonstrated that high reprogramming efficiencies, however, can be more complicated to perform [66].

Interestingly, iPSCs technology has been possible to create disease-specific cells from individual patients that could be analyzed the disease pathology, provided treatment methods and drug development [65]. In addition, it has been documented that iPSCs can be generated into the desired cell types, which hold great promise for treating many diseases. There have been reported in iPSC-derived cells in clinical applications in several types of disease including neurological, immune system, endocrinology/metabolism, muscle skeletal, genetic, hematological, and inherited liver disease [68].

3.2. Differentiation of iPSCs into insulin-producing cells and transplantation

The achievement of hESCs differentiation into insulin-producing cells has raised up the possibility of generation of iPSCs-derived insulin-secreted cells by adopted the same protocols that have been used in hESCs works into iPSCs studies with or without modification the protocols. Recently, researchers have also been differentiated iPSCs into functional insulin-producing cells *in vitro*. The resulting cells expressed pancreatic lineage-related genes, which further normalized blood glucose levels and restored insulin secretion when transplantation into diabetic models. As such, the potential of iPSC-derived insulin-producing cells is also considered as useful tool for diabetes therapy [69–72]. Currently, it has been demonstrated that iPSCs differentiated into posterior foregut and endocrine cells can be transplant and secrete insulin in immunodeficient mouse model [73].

These findings suggested that iPSC-derived insulin-producing cells will provide a potential therapeutic source for treating diabetes. It should be noted that iPSC technology is a possible alternative to induce patient-derived iPSCs for autologous cell transplantation therapy.

3.3. The limitations of hESCs and iPS cells

The potential application of hESCs has been extensively studied due to their high differentiation capacity. However, the use of hESCs to generate functional cells raises the problems associated with technical limitations and ethical issues. These limitations including

(1) the ethical controversies associated with the use of fresh human embryos, (2) developing xenogeneic products-free culture systems both for culturing hESCs and insulin-producing cell differentiation, (3) teratoma formation has become a critical obstacle for the therapeutic applications of hESCs, and (4) destruction of transplanted cells *in vivo*.

The ethical controversies represent another issue associated the use of fresh human embryos. There is concern about guidelines on the use of fresh embryos as a source of new hESC lines.

http://dx.doi.org/10.5772/62850

In this regard, induced pluripotent stem cells (iPSCs) technology provides a solution to the ethical debate surrounding hESCs since it does not require both the destruction of an embryo and the use of human oocytes [74].

It is generally believed that highly purified progenitors or terminally differentiated cell types derived from hESCs results in prevention of teratoma formation [75]. While subpopulations of hESCs have been characterized by the expression of distinct surface markers, their fates have provided a valuable tool for generating tissue-specific reagents for cell-based therapy [76]. Moreover, the combined gene transfer/hESCs therapies can generate a pure population of genetically modified differentiated cells with the selection using lineage-specific markers [77]. Furthermore, the encapsulation procedure has the potential to prevent the formation of tumors [78].

The possible destruction of transplanted hESCs derivatives by the patient's immune system should also be considered before the transplantation of these cells. Some solutions to prevent the rejection of hESC-derived cells are the use of hematopoietic chimerism for tolerance induction and the creation of universal donor cell line [79, 80] and encapsulation technology [81].

The great promise of iPSCs is based on their properties of self-renewal capacity and differentiation into specialized cell types. In addition, the possibility to obtain patient-specific or disease-specific pluripotent stem cells is a promising approach for medical applications. Moreover, iPSCs are not associated with ethical issues as hESCs regarding the use of human oocytes or embryos in research. However, there are still some limitations including inadequate cell number, immune rejection, and teratoma formation upon transplantation.

3.4. Encapsulation technology: a power tool for hESCs and iPS cells applications

In the context of therapeutic applications, the encapsulation technology represents a powerful tool toward the implementation of hESCs and iPSCs in clinical and industrial applications. Generally, there are two types of encapsulation technologies available at the present time including microencapsulation and macroencapsulation. Microencapsulation technology aims to generate a small size semipermeable bag which popularly made of hydrogel polymers to cover small group of cells. On the other hand, macroencapsulation technology has been used to generate semipermeable membrane and hydrogel sheets to hold large scale cell quantity. In the aspect of oxygen diffusion into the encapsulated environment, the microencapsulated sphere allows higher oxygen diffusion capacity than macroencapsulated sphere.

In stem cell research, the encapsulated hESC results in high expansion ratio and high cell recovery yields after cryopreservation. This method also improves the culture of hESC aggregates by protecting cells from hydrodynamic shear stress, controlling aggregate size, and maintaining cell pluripotency [82]. It has been demonstrated that hESCs encapsulated in alginate hydrogels maintain the undifferentiated state and retain their pluripotent capabilities without any enzymatic treatment, mechanical expansion, or manipulation in a feeder-free environment. This approach is well-suited for providing automated culture scale-up process

and the opportunity of long-term culture, feeder-free, and non-labor-intensive culture of hESCs [83].

Alginate encapsulation systems have been shown to support the ability of ES cells to differentiate into specific cell types. The researchers use an alginate encapsulation process for the proliferation and growth of mESC aggregates, which further supports the differentiation of insulin-positive cells from mESCs [84]. In addition, the other group demonstrates 3D model to culture and differentiate hESCs that are encapsulated in calcium alginate microcapsules. This system promotes cellular interactions that are essential for both maintaining pluripotency and differentiation. In addition, encapsulated hESCs are separated from feeder cells during the process of differentiation, which mimics *in vivo* microenvironment and bypass the EBs formation step in a controlled manner. Thus, this 3D culturing of hESCs using alginate microcapsules may be useful for direct differentiation of hESCs toward particular cell types and also has potential for immunoisolation and prevention of teratoma formation of hESCs during transplantation [85].

Cell encapsulation has been proposed to be a solution for treatment of diabetes since it potentially allows the cell protection from host immune system by a concept of immunoisolation. In particular, the microcapsules of islets provide a delicate balance of characteristics including physical strength, immunocompatibility, and selective permeability that will block large immune components. Additionally, its membrane allows the passage of smaller molecules such as oxygen, glucose, water, and insulin [86]. In addition, encapsulated islets in a biocompatible alginates have protected the islets against immune rejection, which is confirmed by prolonged survival of encapsulated islet allografts up to 200 days [87]. Furthermore, Schneider et al. [88] have developed a microcapsule system that protects adult rat and human islets against xenogeneic rejection in immunocompetent diabetic mice without immunosuppression.

3.5. Mechanism of insulin secretion by glucose stimulation

Glucose in the blood is the strong stimulator for the insulin secretion from the islets pancreatic beta cells. Besides glucose, amino acids, ketones, some nutrients, gastrointestinal peptides, and neurotransmitters can also influences the insulin secretion from the beta cells. The glucose-regulated insulin secretion is the complex process and involved several key proteins. Normally, the blood glucose level of more than 70 mg/dl (3.9 mmol/L) enhances insulin production by promoting protein translation and processing. This stimulatory process starts with the binding of glucose to the GLUT2 glucose transporter on the cell membrane of the beta cells. The cytoplasmic glucose is subjected to metabolic process by the action of glucokinase to produce glucose-6-phosphate, which is the rate-limiting step of insulin secretory pathway. Further glycolysis of glucose-6-phosphate finally produces ATP, which is the key molecule for inhibition of the ATP-sensitive K^+ channel protein on the beta cell membrane. The inhibition of ATP-sensitive K^+ channel protein results in beta cell membrane depolarization. This depolarization of the cell membrane induces the opening of voltage-dependent calcium channels with subsequently influx of calcium. High level of cytoplasmic calcium will drive the secretion of insulin from the secretory granules. Insulin secretory profile shows pulsatile

http://dx.doi.org/10.5772/62850

pattern of insulin release, with small bursts occurring about every 10 min. Superimposed upon greater amplitude oscillations of about 80–150 min. This glucose-induced insulin secretion can be enhanced by incretins, released from the neuroendocrine cells of the gastrointestinal tract following food ingestion (**Figure 3**) [89, 90]. However, the mechanism of secretion of insulin with regard to the concentration of glucose from insulin-producing cells derived from hESCs and iPSCs remains unclear.

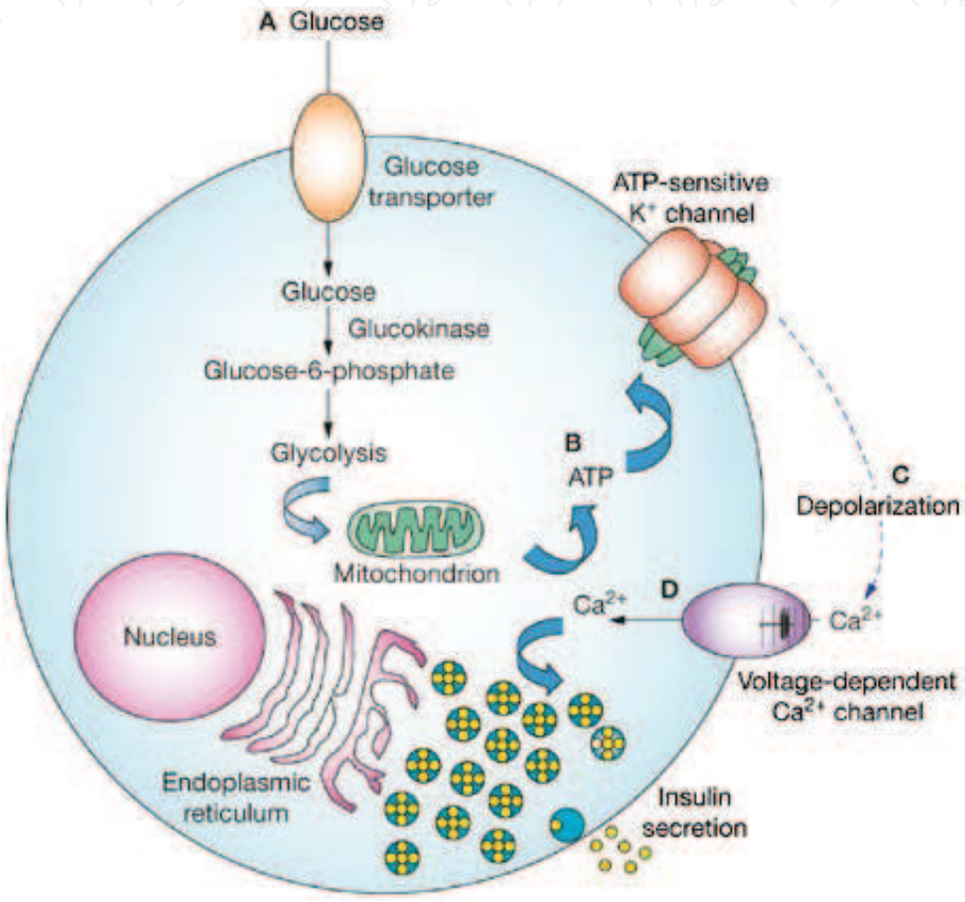

Figure 3. Glucose-stimulated insulin secretion of pancreatic beta cells. In the unstimulated state, the cell ATP-sensitive potassium channels are open, keeping a resting membrane potential of approximately −65 mV. (**A**) Following the uptake of glucose and its metabolism by glucokinase, (**B**) an increase in the intracellular ATP–ADP ratio results in closure of ATP-sensitive potassium channels, (**C**) depolarization of the cell membrane and subsequent opening of voltage-dependent Ca^{2+} channels. (**D**) The resulting increase in cytosolic Ca^{2+} concentration triggers insulin release [90].

4. Conclusion

To date, the researchers have discovered a process that can generate insulin-producing cells from both hESCs and iPSCs. The step toward generating the insulin-producing cells required a differentiation protocol in a manner that mimics differentiation *in vivo*. In order to promote the mature cells, the expression of a key transcription factors should be achieved *in*

vitro. Based on these studies, the differentiation processes were successful at generating functional insulin-producing cells *in vitro*. Although there may be some concerns about hESC and iPSCs research, there are emerging evidences that both hESCs and iPSCs were successfully engrafted, secreted insulin, and regulated blood glucose level in animal models. The encapsulation technology also improves the transplantation efficiency by prevention of encapsulated cells from immune destruction, reduce risks of cancer generation by stem cells, and decrease chronic immunosuppression health risks.

However, current prospects for hESC- and iPSCs-based therapy for diabetes treatment still be requires to investigated further more in early phase and subsequent trials in animal models to generate more safety and effective treatment prior to apply to human therapy.

Author details

Wilairat Leeanansaksiri[1*], Piyaporn Rattananinsruang[1] and Chavaboon Dechsukhum[2]

*Address all correspondence to: wilairat@g.sut.ac.th

1 School of Preclinic, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

2 School of Pathology, Institute of Medicine, Suranaree University of Technology, Nakhon Ratchasima, Thailand

References

[1] Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ. Joslin's diabetes mellitus. 14th ed. Philadelphia: Lippincott Williams & Wilkins, 2005.

[2] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013; 36: S67–S74. [PMID: 23264425 doi:10.2337/dc13-S067]

[3] Mutlu F, Bener A, Eliyan A, Delghan H, Nofal E, Shalabi L, Wadi N. Projection of diabetes burden through 2025 and contributing risk factors of changing disease prevalence: an emerging public health problem. *J Diabetes Metab* 2014; 5: 341–347. [doi: 10.4172/2155-6156.1000341]

[4] Norris SL, Nichols PJ, Caspersen CJ, Glasgow RE, Engelgau MM, Jack L, Jr., Isham G, Snyder SR, Carande-Kulis VG, Garfield S, Briss P, McCulloch D, the Task Force on Community Preventive Services. The effectiveness of disease and case management for people with diabetes. *Am J Prev Med* 2002; 22: 15–38. [PMID: 11985933]

[5] Fowler MJ. Diabetes treatment, part 1: diet and exercise. *Clin Diabetes* 2007; 25: 105–109.

[6] White JR, Davis SN, Davidson MB, Mulcahy K, Manko GA, the Diabetes Consortium Medical Advisory Board. Clarifying the role of insulin in type 2 diabetes management. *Clin Diabetes* 2003; 21: 14–21.

[7] Hirsch IB, Bergenstal RM, Parkin CG, Wright E, Jr., Buse JB. A real-world approach to insulin therapy in primary care practice. *Clin Diabetes* 2005; 23: 78–86.

[8] Qaseem A, Humphrey LL, Chou R, Snow V, Shekelle P, Clinical guidelines committee of the American College of Physicians. Use of intensive insulin therapy for the management of glycemic control in hospitalized patients: a clinical practice guideline from the American College of Physicians. *Ann Intern Med* 2011; 154: 260–267. [PMID: 21320941 doi:10.7326/0003-4819-154-4-201102150-00007]

[9] Bernroider E, Brehm A, Krssak M, Anderwald C, Trajanoski Z, Cline G, Shulman GI, Roden M. The role of intramyocellular lipids during hypoglycemia in patients with intensively treated type 1 diabetes. *J Clin Endocrinol Metab* 2005; 90: 5559–5565. [PMID: 15998784]

[10] Ganiats T. Variability in insulin action: mechanisms, implications, and recent advances. *Internet J Fam Pract* 2006; 5(2):1–9.

[11] Nyenwe EA, Jerkins TW, Umpierrez GE, Kitabchi AE. Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes. *Metabolism* 2011; 60: 1–23. [PMID: 21134520 doi:10.1016/j.metabol.2010.09.010]

[12] Weir GC, Cavelti-Weder C, Bonner-Weir S. Stem cell approaches for diabetes: towards beta cell replacement. *Genome Med*. 2011; 3: 61.

[13] Rother KI, Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. *J Clin Investig* 2004; 114: 877–883. [PMID: 15467822 doi:10.1172/JCI23235]

[14] Ren J, Jin P, Wang E, Liu E, Harlan DM, Li X, Stroncek DF. Pancreatic islet cell therapy for type I diabetes: understanding the effects of glucose stimulation on islets in order to produce better islets for transplantation. *J Transl Med* 2007; 5: 1–15. [PMID: 17201925 doi:10.1186/1479-5876-5-1]

[15] Gunasekaran S. Human pancreatic islet transplantation. *Int J Diabetes Dev Ctries* 2003; 23: 55–57.

[16] Fung M, Thompson D, Warnock G. Pancreatic islet transplantation: a review. *BC Med J* 2004; 46: 457–460.

[17] Pepper AR, Gala-Lopez B, Ziff O, Shapiro AMJ. Current status of clinical islet transplantation. *World J Transpl* 2013; 24: 48–53. [PMID: 24392308 doi:10.5500/wjt.v3.i4.48]

[18] Bruni A, Gala-Lopez B, Pepper AR, Abualhassan NS, Shapiro AMJ. Islet cell transplantation for the treatment of type 1 diabetes: recent advances and future challenges.

Diabetes Metab Syndr Obes 2014; 7: 211–223. [PMID: 25018643 doi:10.2147/DMSO.S50789]

[19] Meier JJ, Bhushan A, Butler PC. The potential for stem cell therapy in diabetes. *Pediatr Res* 2006; 59: 65R–73R. [PMID: 16549551 doi:10.1203/01.pdr.0000206857.38581.49]

[20] Shi Y. Generation of functional insulin-producing cells from human embryonic stem cells in vitro. *Methods Mol Biol* 2010; 636: 79–85. [PMID: 20336517 doi: 10.1007/978-1-60761-691-7_5]

[21] Bose B, Shenoy SP, Konda S, Wangikar P. Human embryonic stem cell differentiation into insulin secreting β-cells for diabetes. *Cell Biol Int* 2012; 36: 1013–1020. [PMID: 22897387 doi:10.1042/CBI20120210]

[22] Skottman H, Hovatta O. Culture conditions for human embryonic stem cells. *Reproduction* 2006; 132: 691–698. [PMID: 17071770]

[23] Kim HS, Oh SK, Park YB, Ahn HJ, Sung KC, Kang MJ, Lee LA, Suh CS, Kim SH, Kim DW, Moon SY. Methods for derivation of human embryonic stem cells. *Stem Cells* 2005; 23: 1228–1233. [PMID: 16051988]

[24] Trounson A. The production and directed differentiation of human embryonic stem cells. *Endocr Rev* 2006; 27: 208–219. [PMID: 16434509]

[25] Stojkovic M, Lako M, Strachan T, Murdoch A. Derivation, growth and applications of human embryonic stem cells. *Reproduction* 2004; 128: 259–267.

[26] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282: 1145–1147.

[27] Mallon BS, Park KY, Chen KG, Hamilton RS, McKay RD. Toward xeno-free culture of human embryonic stem cells. *Int J Biochem Cell Biol* 2006; 38: 1063–1075. [PMID: 16469522]

[28] Cheng L, Hammond H, Ye Z, Zhan X, Dravid, G. Human adult marrow cells support prolonged expansion of human embryonic stem cells in culture. *Stem Cells* 2003; 21: 131–142. [PMID: 12634409]

[29] Richards M, Tan S, Fong CY, Biswas A, Chan WK, Bongso, A. Comparative evaluation of various human feeders for prolonged undifferentiated growth of human embryonic stem cells. *Stem Cells* 2003; 21: 546–556. [PMID: 12968109]

[30] Lee JB, Song JM, Lee JE, Park JH, Kim SJ, Kang SM, Kwon JN, Kim MK, Roh SI, Yoon HS. Available human feeder cells for the maintenance of human embryonic stem cells. *Reproduction* 2004; 128: 727–735. [PMID: 15579590]

[31] Kibschull M, Mileikovsky M, Michael IP, Lye SJ, Nagy A. Human embryonic fibroblasts support single cell enzymatic expansion of human embryonic stem cells in xeno-free cultures. *Stem Cell Res* 2011; 6: 70–82. [PMID: 20934930 doi:10.1016/j.scr.2010.08.002]

[32] Stojkovic P, Lako M, Stewart R, Przyborski S, Armstrong L, Evans J, Murdoch A, Strachan T, Stojkovic M. An autogeneic feeder cell system that efficiently supports growth of undifferentiated human embryonic stem cells. *Stem Cells* 2005; 23: 306–314. [PMID: 15749925]

[33] Li Z, Leung M, Hopper R, Ellenbogen R, Zhang M. Feeder-free self-renewal of human embryonic stem cells in 3D porous natural polymer scaffolds. *Biomaterials* 2010; 31: 404–412. [PMID: 19819007 doi:10.1016/j.biomaterials.2009.09.070]

[34] Lu HF, Narayanan K, Lim SX, Gao S, Leong MF, Wan AC. A 3D microfibrous scaffold for long-term human pluripotent stem cell self-renewal under chemically defined conditions. *Biomaterials* 2012; 33: 2419–2430. [PMID: 22196900 doi:10.1016/j.biomaterials.2011.11.077]

[35] Chen VC, Couture SM, Ye J, Lin Z, Hua G, Huang HI, Wu J, Hsu D, Carpenter MK, Couture LA. Scalable GMP compliant suspension culture system for human ES cells. *Stem Cell Res* 2012; 8: 388–402. [PMID: 22459095 doi:10.1016/j.scr.2012.02.001]

[36] Raikwar SP, Mueller T, Zavazava N. Strategies for developing therapeutic application of human embryonic stem cells. *Physiology (Bethesda)* 2006; 21: 19–28. [PMID: 16443819]

[37] Hwang NS, Varghese S, Elisseeff J. Controlled differentiation of stem cells. *Adv Drug Deliv Rev* 2008; 60: 199–214. [PMID: 18006108]

[38] Kurosawa H. Methods for inducing embryoid body formation: *in vitro* differentiation system of embryonic stem cells. *J Biosci Bioeng* 2007; 103: 389–398. [PMID: 17609152]

[39] Karp JM, Yeh J, Eng G, Fukuda J, Blumling J, Suh KY, Cheng J, Mahdavi A, Borenstein J, Langer R, Khademhosseini A. Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. *Lab Chip* 2007; 7: 786–794. [PMID: 17538722]

[40] Maria R, Nicholas B, Austin S. Towards consistent generation of pancreatic lineage progenitors from human pluripotent stem cells. *Philos Trans R R Soc B* 2015; 370: 1–11.

[41] Camilla HR, Dorthe RP, Jonas BM, Mattias H, Martin D. Collagen type I improve the differentiation of human embryonic stem cells towards definitive endroderm. *Plos One* 2015; 10 (12): 1–21.

[42] Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human embryonic stem cells into insulin-producing clusters. *Stem Cells* 2004; 22: 265–274. [PMID: 15153604]

[43] Jiang J, Au M, Lu K, Eshpeter A, Korbutt G, Fisk G, Majumdar AS. Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells* 2007; 25: 1940–1953. [PMID: 17510217]

[44] Bruin JE, Erener S, Vela J, Hu X, Johnson JD, Kurata HT, Lynn FC, Piret JM, Asadi A, Rezania A, Kieffer TJ. Characterization of polyhormonal insulin-producing cells

derived in vitro from human embryonic stem cells. *Stem Cell Res* 2014; 12: 194–208. [PMID: 24257076 doi:10.1016/j.scr.2013.10.003]

[45] Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes* 2001; 50: 1691–1697. [PMID: 11473026]

[46] Baharvand H, Jafary H, Massumi M, Ashtiani SK. Generation of insulin-secreting cells from human embryonic stem cells. *Dev Growth Differ* 2006; 48: 323–332. [PMID: 16759282]

[47] Jones MP, Burns CJ, Persaud S. Beta-cell replacement technologies: the potential of stem cells. *Drug Discov Today Ther Strateg* 2004; 1: 213–217.

[48] Jacqueline VS, Suzanne JM, Susan Hawes, Andrew GE, Edouard GS. Derivation of insulin-producing beta-cells from human pluripotent stem cells. *Rev Diabetic Stud* 2014; 11: 6–18. [doi:10.1900/RDS.2014.11.6]

[49] Wei R, Yang J, Hou W, Liu G, Gao M, Zhang L, Wang H, Mao G, Gao H, Chen G, Hong T. Insulin-producing cells derived from human embryonic stem cells: comparison of definitive endoderm- and nestin-positive progenitor-based differentiation strategies. *Plos One* 2013; 8: e72513. [PMID: 23951327 doi:10.1371/journal.pone.0072513]

[50] Talitha VDM, Mark OH. Maturation of stem cell-derived beta-cells guided by the expression of Urocortin 3. *Rev Diabet Stud* 2014; 11: 115–132.

[51] Felicia WP, Jeffrey RM, Mads G, Michael S, Alana VD, Jennifer HR, Quinn PP, Dale G, Douglas AM. Generation of functional human pancreatic β cells in vitro. *Cell* 2014; 159: 428–439.

[52] Ahmed E, Nagwa E. The cell cycle as a brake for β-cell regeneration from embryonic stem cells. *Stem cell Res Ther* 2016; 7: 1–9.

[53] Fujikawa T, Oh SH, Pi L, Hatch HM, Shupe T, Petersen BE. Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *Am J Pathol* 2005; 166: 1781–1791. [PMID: 15920163]

[54] Eshpeter A, Jiang J, Au M, Rajotte RV, Lu K, Lebkowski JS, Majumdar AS, Korbutt GS. In vivo characterization of transplanted human embryonic stem cell-derived pancreatic endocrine islet cells. *Cell Prolif* 2008; 41: 843–858. [PMID: 19040565 doi:10.1111/j.1365-2184.2008.00564.x]

[55] Hua XF, Wang YW, Tang YX, Yu SQ, Jin SH, Meng XM, Li HF, Liu FJ, Sun Q, Wang HY, Li JY. Pancreatic insulin-producing cells differentiated from human embryonic stem cells correct hyperglycemia in SCID/NOD mice, an animal model of diabetes. *Plos One* 2014; 10: e102198. [PMID: 25009980 doi:10.1371/journal.pone.0102198]

[56] Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-

responsive insulin-secreting cells *in vivo*. *Nat Biotechnol* 2008; 26: 443–452. [PMID: 18288110 doi:10.1038/nbt1393]

[57] Rezania A, Bruin JE, Riedel MJ, Mojibian M, Asadi A, Xu J, Gauvin R, Narayan K, Karanu F, O'Neil JJ, Ao Z, Warnock GL, Kieffer TJ. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. *Diabetes* 2012; 61: 2016–2029. [PMID: 22740171 doi:10.2337/db11-1711]

[58] Kirk K, Hao E, Lahmy R, Itkin-Ansari P. Human embryonic stem cell derived islet progenitors mature inside an encapsulation device without evidence of increased biomass or cell escape. *Stem Cell Res* 2014; 12: 807–814. [PMID: 24788136 doi:10.1016/j.scr.2014.03.003]

[59] Teoh HK, Cheong SK. Induced pluripotent stem cells in research and therapy. *Malays J Pathol* 2012; 34: 1–13. [PMID: 22870592]

[60] Takahashi K, Yamanaka S. Induced pluripotent stem cells in medicine and biology. *Development* 2013; 140: 2457–2461. [PMID: 23715538 doi:10.1242/dev.092551]

[61] Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nat Cell Biol* 2013; 13: 497–505. [PMID: 21540845 doi:10.1038/ncb0511-497]

[62] Lin SL, Chang DC, Ying SY, Leu D, Wu DT. MicroRNA miR-302 inhibits the tumorigenecity of human pluripotent stem cells by coordinate suppression of the CDK2 and CDK4/6 cell cycle pathways. *Cancer Res* 2010; 70: 9473–9482. [PMID: 21062975 doi:10.1158/0008-5472.CAN-10-2746]

[63] Kuo CH, Ying SY. Advances in microRNA-mediated reprogramming technology. *Stem Cells Int* 2012; 2012: 823709. [PMID: 22550519 doi:10.1155/2012/823709]

[64] Rosa A, Brivanlou AH. Regulatory non-coding RNAs in pluripotent stem cells. *Int J Mol Sci* 2013; 14: 14346–14373. [PMID: 23852015 doi:10.3390/ijms140714346]

[65] Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ. Disease-specific induced pluripotent stem cells. *Cell* 2008; 134: 877–886. [PMID: 18691744 doi:10.1016/j.cell.2008.07.041]

[66] Zhou Y, Zeng F. Integration-free methods for generating induced pluripotent stem cells. *Genom Proteom Bioinform* 2013; 11: 284–287. [doi:10.1016/j.gpb.2013.09.008]

[67] Stadtfeld M, Hochedlinger K. Induced pluripotency: history, mechanisms, and applications. *Genes Dev* 2010; 24: 2239–2263. [PMID: 20952534 doi:10.1101/gad.1963910]

[68] Chun YS, Chaudhari P, Jang YY. Applications of patient-specific induced pluripotent stem cells; focused on disease modeling, drug screening and therapeutic potentials for liver disease. *Int J Biol Sci* 2010; 6: 796–805. [PMID: 21179587]

[69] Zhu FF, Zhang PB, Zhang DH, Sui X, Yin M, Xiang TT, Shi Y, Ding MX, Deng H. Generation of pancreatic insulin-producing cells from rhesus monkey induced

pluripotent stem cells. *Diabetologia* 2011; 54: 2325–2336. [PMID: 21755313 doi:10.1007/s00125-011-2246-x]

[70] Jeon K, Lim H, Kim JH, Thuan NV, Park SH, Lim YM, Choi HY, Lee ER, Kim JH, Lee MS, Cho SG. Differentiation and transplantation of functional pancreatic beta cells generated from induced pluripotent stem cells derived from a type 1 diabetes mouse model. *Stem Cells Dev* 2012; 21: 2642–2655. [PMID: 22512788 doi:10.1089/scd.2011.0665]

[71] Wang L, Huang Y, Guo Q, Fan X, Lu Y, Zhu S, Wang Y, Bo X, Chang X, Zhu M, Wang Z. Differentiation of iPSCs into insulin-producing cells via adenoviral transfection of PDX-1, NeuroD1 and MafA. *Diabetes Res Clin Pract* 2014; 104: 383–392. [PMID: 24794627 doi:10.1016/j.diabres.2014.03.017]

[72] Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'Dwyer S, Quiskamp N, Mojibian M, Albrecht T, Yang YH, Johnson JD, Kieffer TJ. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol* 2014; 32(11): 1121–1133. [doi:10.1038/nbt.3033]

[73] Pellegrini S, Ungaro F, Mercalli A, Melzi R, Sebastiani G, Dotta F, Broccoli V, Piemonti L, Sordi V. Human induced pluripotent stem cells differentiation into insulin-producing cells able to engraft in vivo. *Acta Diabetol*. 2015; 52(6): 1025–1035. [doi:10.1007/s00592-015-0726-z]

[74] Kastenberg ZJ, Odorico JS. Alternative sources of pluripotency: science, ethics, and stem cells. *Transplant Rev (Orlando)* 2008; 22: 215–222. [PMID: 18631882 doi:10.1016/j.trre.2008.04.002]

[75] Hentze H, Soong PL, Wang ST, Phillips BW, Putti TC, Dunn NR. Teratoma formation by human embryonic stem cells: evaluation of essential parameters for future safety studies. *Stem Cell Res* 2009; 2: 198–210. [PMID: 19393593 doi:10.1016/j.scr.2009.02.002]

[76] King FW, Ritner C, Liszewski W, Kwan HC, Pedersen A, Leavitt AD, Bernstein HS. Subpopulations of human embryonic stem cells with distinct tissue-specific fates can be selected from pluripotent cultures. *Stem Cells Dev* 2009; 18: 1441–1450. [PMID: 19254177 doi:10.1089/scd.2009.0012]

[77] Strulovici Y, Leopold PL, O'Connor TP, Pergolizzi RG, Crystal RG. Human embryonic stem cells and gene therapy. *Mol Ther* 2007; 15: 850–866. [PMID: 17356540]

[78] Fong CY, Gauthaman K, Bongso A. Teratomas from pluripotent stem cells: a clinical hurdle. *J Cell Biochem* 2010; 111: 769–781. [PMID: 20665544 doi:10.1002/jcb.22775]

[79] Drukker M, Benvenisty N. The immunogenicity of human embryonic stem-derived cells. *Trends Biotechnol* 2004; 22: 136–141. [PMID: 15036864]

[80] Boyd AS, Higashi Y, Wood KJ. Transplanting stem cells: potential targets for immune attack. Modulating the immune response against embryonic stem cell transplantation. *Adv Drug Deliv Rev* 2005; 57: 1944–1969. [PMID: 16289432]

[81] Arturo JV, Omid V, Mads G, Jeffrey RM, Felicia WP, Andrew RB, Joshua CD, Jie L, Michael C, Karsten O, Hok HT, Siddharth J, Erin L, Stephanie A, Srujan G, James JM, Matthew AB, Jennifer H, Jose O, Dale LG, Gordon CW, Douglas AM, Robert L, Daniel GA. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat Med* 2016; 22: 306–311[doi:10.1038/nm.4030]

[82] Serra M, Correia C, Malpique R, Brito C, Jensen J, Bjorquist P, Carrondo MJ, Alves PM. Microencapsulation technology: a powerful tool for integrating expansion and cryopreservation of human embryonic stem cells. *Plos One* 2011; 6: e23212. [PMID: 21850261 doi:10.1371/journal.pone.0023212]

[83] Siti-Ismail N, Bishop AE, Polak JM, Mantalaris A. The benefit of human embryonic stem cell encapsulation for prolonged feeder-free maintenance. *Biomaterials* 2008; 29: 3946–3952. [PMID: 18639332 doi:10.1016/j.biomaterials.2008.04.027]

[84] Wang N, Adams G, Buttery L, Falcone FH, Stolnik S. Alginate encapsulation technology supports embryonic stem cells differentiation into insulin-producing cells. *J Biotechnol* 2009; 144: 304–312. [PMID: 19686786 doi:10.1016/j.jbiotec.2009.08.008]

[85] Chayosumrit M, Tuch B, Sidhu K. Alginate microcapsule for propagation and directed differentiation of hESCs to definitive endoderm. *Biomaterials* 2010; 31: 505–514. [PMID: 19833385 doi:10.1016/j.biomaterials.2009.09.071]

[86] Lee MK, Bae YH. Cell transplantation for endocrine disorders. *Adv Drug Deliv Rev* 2000; 42: 103–120. [PMID: 10942817]

[87] de Vos P, Faas MM, Strand B, Calafiore R. Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials* 2006; 27: 5603–5617. [PMID: 16879864]

[88] Schneider S, Feilen PJ, Brunnenmeier F, Minnemann T, Zimmermann H, Zimmermann U, Weber MM. Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent diabetic mice. *Diabetes* 2005; 54: 687–693. [PMID: 15734844]

[89] Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine 19/E 19th Edition. McGraw-Hill Medical, New York, USA, 2015.

[90] De León DD, Stanley CA. Mechanisms of disease: advances in diagnosis and treatment of hyperinsulinism in neonates. *Nat Clin Pract Endocrinol Metab* 2007; 3: 57–68. [doi:10.1038/ncpendmet0368]

IntechOpen